09/743905

U.S. Application No. Pending | International Application No. PCT/BE99/00089 | Attorney Docket No. VANM190.001APC

Date: January 10, 2001

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/BE99/00089
International Filing Date:	July 9, 1999
Priority Date Claimed:	July 10, 1998
Title of Invention:	METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL SEQUENCE
Applicant(s) for DO/EO/US:	E. Lauber, Hubert Guilley, Ken Richards, Gerard Jonard

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) () is transmitted herewith (required only if not transmitted by the International Bureau).
   b) (X) has been transmitted by the International Bureau.
   c) () is not required, as the application was filed in the United States Receiving Office (RO/US).

5. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))

   a) () are transmitted herewith (required only if not transmitted by the International Bureau).
   b) () have been transmitted by the International Bureau.
   c) () have not been made; however, the time limit for making such amendments has NOT expired.
   d) (X) have not been made and will not be made.

6. (X) A FIRST preliminary amendment, includes 1-page Abstract.

7. (X) International Application as published.
   a. (X) Publication Cover Sheet
   b. (X) 28 pages of disclosure
   c. (X) International Search Report

8. (X) PCT Form PCT/IPEA/402.

9. (X) PCT Form PCT/IB/308.

10. (X) A return prepaid postcard.

11. (X) The following fees are submitted:

				FEES
	BASIC FEE			$860
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	26 - 20 =	6 ×	$18	$108
Independent Claims	1 - 3 =	0 ×	$80	$0
	TOTAL OF ABOVE CALCULATIONS		$968	
	TOTAL NATIONAL FEE			$968

12. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.

13. (X) A check in the amount of $968 to cover the above fees is enclosed.

14. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Signature

Daniel E. Altman
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34,115
Registration Number

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011001

09/743,905

371 filing date-April 24, 2001

APPLICATION DOES NOT COMPLY WITH THE SEQUENCE RULES. SEE REASONS BELOW.

A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the *Federal Register* at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and where applicable, includes no new matter, as required by 37 C.F.R. 1.821(e), 1.821(f), 1.821(g), 1.825(b) and 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the Patent and Trademark Office, such request in accordance with 37 C.F.R. 1.821(e) may be submitted in lieu of a new CRF.

VANM190.001APC

PATENT

#6a Date 7/11/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Lauber, E.	Group Art Unit Unknown
Appl. No.	:	PCT/BE99/00089	
Filed	:	July 9, 1999	
For	:	METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL SEQUENCE	
Examiner	:	Unknown	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to Examination on the merits, please amend the above-captioned patent application as follows:

IN THE SPECIFICATION

On page 1, line 11, before the Field of the Invention, please insert --This is the U.S. National Phase under 35 U.S.C. §371 of International Patent Application PCT/BE99/00089, filed July 9, 1999, which claims priority of European application EP 98870159.5, filed July 10, 1998--.

On page 2, line 7, please cancel the word "strategy" and substitute in its place --strategies--.

On page 3, line 10, before the word "resistance" and after the word "as", please insert --a--.

On page 3, line 18, please cancel the word "completely".

On page 3, line 19, after the word "mechanisms" and before the ".", please insert the word --completely--.

On page 12, line 15, please cancel the word "into" and substitute in its place --in--.

On page 12, line 19, please cancel the word "into" and substitute in its place --in--.

On page 12, line 23, please cancel the word "into" and substitute in its place --in--.

On page 23, line 1, please cancel the word "CLAIMS" and substitute in its place -- WHAT IS CLAIMED IS--.

IN THE ABSTRACT

Please insert the enclosed page 29.

IN THE CLAIMS

Please amend the claims as follows:

1. **(Amended)** A **[Method]**method of **[genetic modification of a TGB-3 wild type viral sequence for reducing or suppressing the possible deleterious effects of the agronomic properties of a transformed plant or plant cell by said]** identifying mutants in a TGB-3 viral sequence which inhibit infection of a virus into a cell, comprising **[the following successive steps]**:

- **[submitting]**mutating said TGB-3 sequence **[to point mutation(s) which allow the substitution of at least one amino-acid into a different amino-acid,]**;
- selecting **[genetically modified]** TGB-3 mutants**[wild type viral sequences having said point mutation(s) and]** which no longer **[are not able to]** promote cell-to-cell movement of a (TGB-3 minus) mutant virus **[having a dysfunctional TGB-3 wild type viral sequence,]** when expressed in trans from a replicon**[,]**;
- further selecting from the identifed mutants**[among said genetically modified TGB-3 viral sequences, the specifically genetically modified sequence]**those which also inhibit**[s]** infection with a co-inoculated wild

type virus when the **[mutant form was]**mutant TGB-3 is expressed from a replicon**[,]**; and

recovering said **[specifically genetically modified]**mutant TGB-3 viral sequence.

2. **(Amended)** The **[Method]**method according to Claim 1, wherein the TGB-3 wild type viral sequence is the BNYVV P15 sequence.

3. **(Amended)** **[Genetically]**A genetically modified TGB-3 viral sequence obtained by the method according to Claim 1**[or 2]**.

4. **(Amended)** **[Genetically]**The genetically modified TGB-3 viral sequence according to Claim 3, **[being]** selected from the group consisting of **[the following sequences]**:

[SEQ ID NO 1:

```
ATGGTGCTTGTGGTTGCAGTAGCTTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M  V  L  V  V  A  V  A  L  S  N  I  V  L  Y  I  V  A  G  C
GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V  V  V  S  M  L  Y  S  P  F  F  S  N  D  V  K  A  S  S  Y
GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
A  G  A  I  F  K  G  S  G  C  I  M  D  R  N  S  F  A  Q  F
GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G  S  C  D  I  P  K  H  V  A  E  S  I  T  K  V  A  T  K  E
CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H  D  V  D  I  M  V  K  R  G  E  V  T  V  R  V  V  T  L  T
GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360
E  T  I  F  I  I  L  S  R  L  F  G  L  A  V  F  L  F  M  I
TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
```

C L M S I V W F W Y H R *

SEQ ID NO 2:

ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60

M V L V V K V D L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120

V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGCCGCGAATTCGTTTGCTCAATTT 180

A G A I F K G S G C I M A A N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240

G S C D I P K H V A E S I T K V A T K E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300

H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360

E T I F I I L S R L F G L A V F L F M I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399

C L M S I V W F W Y H R *

SEQ ID NO 3:

ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60

M V L V V K V D L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120

V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180

A G A I F K G S G C I M D R N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240

G S C D I P K H V A E S I T K V A T K E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300

H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGATGATTTTTTGTTCATGATA 360

E T I F I I L S R L F G L D D F L F M I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399

C L M S I V W F W Y H R *] SEQ ID NOS:1, 3, and 5.

5. **(Amended)** **[Vector]**A vector comprising the genetically modified TGB-3 viral sequence according to **[the]** Claim 3 **[or 4, possibly linked to one or more regulatory sequence(s) capable of being active into a plant or a plant cell]**.

6. **(Amended)** **[Method]**A method for inducing resistance **[into]**to a virus in a plant or a plant cell **[to a virus]** comprising **[a TGB-3 sequence, comprising the following steps]**:

- preparing a nucleic acid construct comprising a genetically modified TGB-3 viral sequence according to Claim **[4 or 5, being]**3 operably linked to one or more regulatory sequence(s) **[capable of being]** active **[into]**in a plant or a plant cell, and
- transforming a plant cell with said nucleic acid construct**[, and possibly**
- **regenerating a transgenic plant from the transformed plant cell]**.

7. **(Amended)** **[Method]**The method according to Claim 6, **[characterised in that]**wherein the virus is selected from the group consisting of the apple stem pitting virus, the blueberry scorch virus, the potato virus M, the white clover mosaic virus, the *Cymbidium* mosaic virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus, the beet soil-borne virus **[or]**and the BNYVV virus.

8. **(Amended)** **[Method]**The method according to Claim 6 **[or 7, characterised in that]**wherein the plant cell is a stomatal cell.

9. **(Amended)** **[Method]**The method according to **[any one of the Claims 6 to 8, characterised in that]**Claim 6 wherein the plant is selected from the group consisting of apple, blueberry, potato, clover, orchid, barley, peanut **[or]**and sugar beet.

10. **(Amended)** **[Method]**The method according to **[any one of the Claims 6 to 9, characterized in that]**Claim 6, wherein the regulatory sequence comprises a promoter sequence or a terminator sequence active in a plant.

11. **(Amended)** **[Method]**The method according to Claim 10, **[characterised in that]**wherein the promoter sequence is a constitutive or a foreigner promoter sequence.

12. **(Amended)** **[Method]**The method according to Claim 10, **[characterised in that]**wherein the promoter sequence is selected from the group consisting of the 35S Cauliflower Mosaic Virus promoter, **[and/or]** the polyubiquitin Arabidopsis thaliana promoter, and both promoters.

13. **(Amended)** **[Method]**The method according to **[any one of the Claims 10 to 12, characterized in that]**Claim 10, wherein the promoter sequence is a promoter **[which is capable of being]** active **[mainly into]**in the root tissue of plants **[such as the par promoter of the haemoglobin gene from Perosponia andersonii]**.

14. **(Amended)** **[Transgenic]**A transgenic plant or transgenic plant cell resistant to a virus **[and]** comprising a nucleic acid construct having a genetically modified TGB-3 viral sequence according to Claim 4 **[or 5, being]** operably linked to one or more regulatory sequence(s) active **[into]**in a plant or a plant cell.

15. **(Amended)** **[Transgenic]**A transgenic plant or transgenic plant cell according to Claim 14, **[characterised in that]**wherein the virus is selected from the group consisting of the apple stem pitting virus, the blueberry scorch virus, the potato virus M, the white clover mosaic virus, the *Cymbidium* mosaic virus, the potato virus X, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus, the beet soil-borne virus and the BNYVV virus.

16. **(Amended)** **[Transgenic]**The transgenic plant or transgenic plant cell according to Claim 14 **[or 15, being a plant or a plant cell]** selected from the group consisting of apple, blueberry, potato, clover, orchid, barley, peanut **[or]**and sugar beet **[plant or plant cell]**.

17. **(Amended)** **[Transgenic]**The transgenic plant or transgenic plant cell according to **[any one of the Claims 14 to 16, characterised in that]**Claim 14, wherein the regulatory sequence comprises a promoter sequence and a terminator sequence **[capable of being]** active **[into]**in a plant.

18. **(Amended)** **[Transgenic]**The transgenic plant or transgenic plant cell according to **[any one of the Claims 14 to 17, characterised in that]**Claim 14, wherein the regulatory sequence(s) comprise a promoter sequence which is a constitutive or a **[foreigner]** foreign vegetal promoter sequence.

19. **(Amended)** **[Transgenic]**The transgenic plant or transgenic plant cell according to Claim 18, **[characterised in that]**wherein the promoter sequence is selected from the group consisting of the 35S Cauliflower Mosaic Virus promoter, **[and/or]** the polyubiquitin Arabidopsis thaliana promoter, and both.

20. **(Amended)** **[Transgenic]**The transgenic plant or transgenic plant cell according to Claim 18 **[or 19, characterised in that]**wherein the promoter sequence is **[a**

promoter which is mainly] active in root tissues **[such as the par promoter of the haemoglobin gene from Perosponia andersonii]**.

21. **(Amended)** **[Transgenic]**The transgenic plant tissue of Claim 14 wherein said tissue is selected from the group consisting of fruit, stem, root, tuber, and seed **[of a plant according to any one of the Claims 14 to 20]**.

22. **(Amended)** **[Reproducible]**A reproducible structure obtained from a transgenic plant according to **[any one of the Claims 14 to 21]**Claim 14.

Please add the following Claims

23. The vector of Claim 5 operably linked to one or more regulatory sequence(s) active in a plant cell.

24. The method of Claim 5 further comprising regenerating a transgenic plant from the transformed plant cell.

25. The method of Claim 13, wherein said promoter active in the root tissue of plants is the par promoter of the haemoglobin gene from *Perosponia andersonii*.

26. The transgenic plant of Claim 16, wherein said promoter active in the root tissue of plants is the par promoter of the haemoglobin gene from *Perosponia andersonii*.

REMARKS

The specification and claims have been amended and an abstract added to conform with the rules of practice before the United States Patent and Trademark Office. Claims 23-26 have been added. Support for the added claims can be found in the claims as filed. No new matter has been added herewith. As a result of the amendment, Claims 1-26 are presented for prosecution.

Conclusion

Should there be any questions concerning the application, the Examiner is invited to contact the undersigned attorney at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 10 Jan. 2001

By: Daniel Altman

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METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL SEQUENCE

Field of the invention

The present invention is related to a method of genetic modification of a wild type viral sequence, for reducing or suppressing deleterious properties of plants or plant cells transformed by said wild type viral sequence.

The present invention is also related to the modified viral sequence obtained by said method, and to the plant and the plant cell comprising said modified viral sequence.

Background of the invention and state of the art

The widespread viral disease of the sugar beet plant (*Beta vulgaris*) called Rhizomania is caused by a furovirus, the beet necrotic yellow vein virus (BNYVV) (1, 2) which is transmitted to the root of the beet by the soilborne fungus *Polymyxa betae* (3).

The disease affects significantly acreages of the area where the sugar beet plant is grown for industrial use in Europe, USA and Japan and is still in extension in several places in Western Europe (4, 5).

Since 1986, number of reports and publications have described the use of isolated viral nucleotidic sequences expressed in plants to confer a high level of tolerance against a specific infectious virus or even to confer a broad spectrum type of resistance against a number of related viruses (6, 7, 8). One of the most documented viral resistance ~~strategy~~ strategies based on genetic engineering, in many cultivated species such as potato, squash, cucumber or tomato, is the use of the viral nucleotidic sequence which under the control of plant regulatory elements, encodes the coat-protein of the target virus (9).

However, in coat-protein mediated resistance, the expression of a certain level of resistance in the transgenic plant might be attributed to different mechanisms such as RNA co-suppression and not necessarily to the production of the protein sequence.

In general, the virus sequence will be transformed in an appropriate cell or tissue culture of the plant species using an *Agrobacterium* mediated transformation system or a direct gene transfer method according to the constraints of the tissue culture or cell culture method which can be successfully applied in a given species. A whole plant will be regenerated and the expression of the transgene will be characterised.

Though sugar beet is known as a recalcitrant species in cell culture, limiting the extent of practical genetic engineering applications in that species, there are number of isolated reports of successful transformation and regeneration of whole plants (38). A few examples of engineering tolerance to the BNYVV by transforming and expressing the BNYVV coat-protein sequence in the sugar

beet genome have also been published (11, WO91/13159) though they rarely report data on whole functional transgenic sugar beet plants (12). In particular, reports show limited data on the level of resistance observed in infected conditions with transgenic sugar beet plants transformed with a gene encoding a BNYVV coat-protein sequence (13, 14).

A complete technology package including a sugar beet transformation method and the use of the expression of the BNYVV coat-protein sequence as resistance source in the transgenic sugar beet plant obtained by said transformation method has been described in the Patent Application WO91/13159.

Based on the information published, it can not be concluded that the coat-protein mediated resistance mechanism provides any potential for conferring to the sugar beet plant a total immunity to the BNYVV-infection by inhibiting ~~completely~~ Completely the virus multiplication and diffusion mechanisms. To identify a resistance mechanism which significantly blocks the spread of the virus at the early stage of the infection process would be a major criteria of success to develop such a transgenic resistance. In addition, such resistance would diversify the mechanisms of resistance available.

Because the disease is shown to expand in many countries or areas, at a speed depending upon the combination of numerous local environmental and agricultural factors, there is a major interest to diversification and improvement of the genetic resistance mechanisms which may, alone or in combination, confer a stable and long lasting resistance strategy in the current

and future varieties of sugar beet plants which are grown for industrial use.

The genome of beet necrotic yellow vein furovirus (BNYVV) consists of five plus-sense RNAs, two of which (RNAs 1 and 2) encode functions essential for infection of all plants while the other three (RNAs 3, 4 and 5) are implicated in vector-mediated infection of sugar beet (*Beta vulgaris*) roots. Cell-to-cell movement of BNYVV is governed by a set of three successive, slightly overlapping viral genes on RNA 2 known as the triple gene block (TGB), which encode, in order, the viral proteins P42, P13 and P15 (gene products are designated by their calculated M_r in kilodalton).

In the following description, the TGB genes and the corresponding proteins will be identified by the following terms : TGB-1, TGB-2, TGB-3 or by their encoded viral protein number P42, P13 and P15. TGB counterparts are present in other furoviruses and in potex-, carla- and hordeiviruses (15, 18, 19, 20, 21 and 22). In the enclosed table 1 are represented viruses having a TGB-3 sequence, the molecular weight of TGB-3 of said viruses, their host and references.

It has been shown previously that independent expression of P15 from a viral-RNA replication species known as a "replicon", derived from BNYVV RNA 3, inhibits infection with BNYVV by interfering cell-to-cell movement (16).

In order to introduce a virus comprising a TGB-3 nucleic acid sequence into a plant cell or a plant, it has been proposed to incorporate a nucleic acid construct comprising said TGB-3 nucleic acid sequence

operably linked to one or more regulatory sequences active in said plant (WO98/07875).

However, while expression of wild type TGB-3 viral sequence in a transgenic plant allows the blocking of said viral infection, the presence of said wild type sequence may induce deleterious effects on the agronomic properties of transformed plants or plant cells.

Aims of the invention

The present invention aims to provide a new method for inducing a genetic modification of a wild type viral sequence involved in the multiplication and diffusion mechanisms of virus infecting plants, in order to reduce or suppress the possible deleterious effects upon plants or plant cells transformed by said viral sequence.

Another aim of the present invention is to provide a method to obtain such a modified viral sequence which blocks virus infection when it is incorporated into a plant or a plant cell.

Summary of the invention

The present invention is related to a method of genetic modification of a TGB-3 wild type viral sequence, preferably the BNYVV P15 viral sequence, for reducing or suppressing the possible deleterious effects on the agronomic properties of the transformed plants or plant cells by said TGB-3 viral sequence.

Preferably, said genetic modification is a point mutation which allows the substitution of at least one amino-acid into another different amino-acid of said TGB-3 wild type sequence, preferably the substitution of at

least one amino-acid into another different amino-acid in the BNYVV P15 sequence.

It seems that the function of the TGB-3 wild type sequence in cell-to-cell movement involves at least in part "bridging" interactions between an element of the host plant (preferably a component of the plasmodesmata), and an element of viral origin (preferably another viral protein involved in cell-to-cell movement). Disruption of either the domain of the TGB-3 wild type sequence (which putatively interacts with the host element) or the domain of the TGB-3 wild type sequence (which putatively interacts with the viral element), allows the inhibition of the cell-to-cell movement.

In addition, it seems that said specific mutations in a TGB-3 wild type sequence allow the production of mutants produced in a transgenic plant, which will still interact with the viral element, but not with the host element. These mutants might compete for binding sites on the viral element of the TGB-3 wild type sequence produced in the initial stage of the viral infection, and abort the infection by inhibiting viral movement to an adjacent cell.

Advantageously, the substitution of at least one amino-acid into another different amino-acid of said sequence is made in regions rich in hydrophilic amino-acids usually present at the surface of the protein in its native configuration.

Preferably, the point mutation(s) allow the substitution of one or two amino-acids into one or two different amino-acids.

In the enclosed Table 1, preferred examples of said viruses having a TGB-3 wild type viral sequence,

the molecular weight of the corresponding TGB-3 peptide, their hosts and a reference, are described. The specific wild type P15 nucleotidic and amino-acid sequences of BNYVV are also already described (17).

The above-described point mutations were realised by conventional methods known by the person skilled in the art.

The above mutants containing the point mutation were tested for their ability to promote cell-to-cell movement of a viral mutant (with a dysfunctional TGB-3 sequence, preferably a BNYVV mutant with a dysfunctional P15 gene) when expressed in trans from a replicon. These mutants were incapable of promoting such movement and were tested for their ability to inhibit infection with a co-inoculated wild type TGB-3 virus, preferably co-inoculated with a wild type BNYVV, when the mutant form of the TGB-3 sequence, preferably the P15 gene, was expressed from a replicon.

The Inventors have discovered unexpectedly that the genetic modification method according to the invention (preferably a point mutation) could be used to obtain a modified TGB-3 viral sequence (preferably a modified BNYVV P15 sequence), which is able to block virus infection without producing deleterious effects when incorporated in the genome of a plant or a plant cell.

It is meant by "being able to block viral infection into a plant or a plant cell", the possibility to obtain a high degree of tolerance by the plant or plant cell transformed by said modified TGB-3 viral sequence to said viral infection, in particular the possibility to ensure rapid and total blocking of the virus multiplication and diffusion mechanisms into the plant, preferably the

blocking of the BNYVV virus multiplication and diffusion mechanisms into a sugar beet plant (beta vulgaris), including fodder beet, Swiss Whard and table beet which may also be subjected to said BNYVV infection.

Said tolerance or resistance could be easily measured by various methods well known by the person skilled in the art.

Preferably, the genetic modifications in the TGB-3 wild type viral sequence are point mutations in the portions of said wild type viral sequence involved in the mechanisms of viral cell-to-cell movements.

The present invention is also related to the modified TGB-3 viral nucleotidic and amino-acid sequences obtained (recovered) by said (modification and selection) method, more preferably the BNYVV P15 modified nucleotidic and amino-acid sequences obtained (recovered) by said method.

Preferably, said BNYVV P15 nucleotidic and amino-acid sequences are selected from the group consisting of the following nucleotidic or corresponding amino-acid sequences :

SEQ ID NO 1 :

ATGGTGCTTGTGGTTGCAGTAGCTTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M V L V V A V A L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
A G A I F K G S G C I M D R N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G S C D I P K H V A E S I T K V A T K E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360
E T I F I I L S R L F G L A V F L F M I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
C L M S I V W F W Y H R *

SEQ ID NO 2 :

ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M V L V V K V D L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGCCGCGAATTCGTTTGCTCAATTT 180
A G A I F K G S G C I M A A N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G S C D I P K H V A E S I T K V A T K E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360
E T I F I I L S R L F G L A V F L F M I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
C L M S I V W F W Y H R *

SEQ ID NO 3 :

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ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M  V  L  V  V  K  V  D  L  S  N  I  V  L  Y  I  V  A  G  C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V  V  V  S  M  L  Y  S  P  F  F  S  N  D  V  K  A  S  S  Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
A  G  A  I  F  K  G  S  G  C  I  M  D  R  N  S  F  A  Q  F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G  S  C  D  I  P  K  H  V  A  E  S  I  T  K  V  A  T  K  E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H  D  V  D  I  M  V  K  R  G  E  V  T  V  R  V  V  T  L  T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGATGATTTTTTGTTCATGATA 360
E  T  I  F  I  I  L  S  R  L  F  G  L  D  D  F  L  F  M  I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
C  L  M  S  I  V  W  F  W  Y  H  R  *
```

In the following description, the various modified BNYVV TGB-3 sequences will be hereafter called "P15 mutants", identified by the following reference : BNP15-Ala1, corresponding to SEQ ID NO 1, BNP15-Ala4 corresponding to SEQ ID NO 2, BNP15-Asp9, corresponding to SEQ ID NO 3.

The nucleotidic and corresponding amino-acid sequences of SEQ ID NO 1, SEQ ID NO 2 and SEQ ID NO 3 can be compared to SEQ ID NO 4, which is the sequence of the wild type P15 nucleotidic and amino-acid sequence already described (17).

The present invention is also related to the vector comprising said modified nucleotidic sequence possibly being operably linked to one or more regulatory sequence(s) active into a plant or a plant cell. Preferably, said vector is a plasmid comprising already said regulatory sequence(s) active into a plant or a plant cell.

The present invention is also related to a method for inducing a resistance to a virus comprising a TGB-3 sequence, preferably one of the viruses described in the enclosed Table 1, and more preferably the BNYVV virus, said method comprising the following steps :

- preparing a nucleic acid construct comprising a nucleic acid sequence being genetically modified according to the method of the invention and being operably linked to one or more regulatory sequences active into a plant or a plant cell,
- transforming the plant cell with the nucleic acid construct, and
- possibly regenerating the transgenic plant from the transformed plant cell.

Preferably, said method is used for inducing a resistance to the BNYVV into a sugar beet plant or a sugar beet cell. Said method comprises the following steps :

- preparing a nucleic acid construct comprising a modified nucleic acid sequence obtained by the method according to the invention, preferably preparing a nucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 2 or

SEQ ID NO 3, being operably linked to one or more regulatory sequences active into a plant,

- transforming the sugar beet plant cell with the nucleic acid construct, and
- possibly regenerating the transgenic sugar beet plant from the transformed sugar beet plant cell.

The present invention is also related to the obtained (recovered) transgenic plant or the transgenic plant cell resistant to an infection by a virus comprising a TGB-3 sequence, preferably one of the viruses described in the enclosed Table 1, more preferably the BNYVV virus, said plant or plant cell comprising a nucleic acid construct having a TGB-3 modified nucleic acid sequence, being operably linked to one or more regulatory sequences capable of being active into a plant or a plant cell.

Preferably, said modified nucleic acid sequence is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 2 and SEQ ID NO 3, being operably linked to one or more regulatory sequences active into a plant or a plant cell.

Preferably, the cell is a stomatal cell and the regulatory sequence comprises a promoter sequence and a terminator sequence capable of being active into a plant. Said promoter sequence can be constitutive or could be obtained from a foreigner promoter sequence, and is preferably selected from the group consisting of the 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin Arabidopsis thaliana promoter.

Advantageously, the promoter sequence is a promoter which is mainly capable of being active in the root tissue of plants such as the par promoter or the haemoglobin gene from Perosponia andersonii.

A last aspect of the present invention is related to a transgenic plant tissue such as fruit, stem, root, tuber, seed of the transgenic plant according to the invention or a reproducible structure (preferably selected from the group consisting of calluses, buds or embryos) obtained from the transgenic plant or the plant cell according to the invention.

The techniques of plant transformation, tissue culture and regeneration used in the method according to the invention are the ones well known by the person skilled in the art. Such techniques are preferably the ones described in the International Patent Applications WO95/101778, WO91/13159 (corresponding to the European Patent Application EP-B-0517833), WO98/07875, which are incorporated herein by reference.

These techniques are preferably used for the preparation of transgenic sugar beet plants and plant cells according to the invention.

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Table 1

Virus	Size of TGB-3	Host	Reference
Apple stem pitting virus	8 kDa	apple	Jelkman, *J. Gen. Virol.* 75, 1535-1542 (1994)
Blueberry scorch virus	7 kDa	blueberry	Cavileer et al., *J. Gen. Virol.* 75, 711-720 (1994)
Potato virus M	7 kDa	potato	Zavriev et al., *J. Gen. Virol.* 72, 9-14 (1991)
White clover mosaic virus	8 kDa	clover	Forster et al., *Nucl. Acids Res.* 16, 291-303 (1988)
Cymbidium mosaic virus	10 kDa	orchid	Neo et al., *Plant Mol. Biol.* 18, 1027-1029 (1992)
Potato virus X	8 kDa	potato	Rupasov et al., *J. Gen. Virol.* 70, 1861-1869 (1994)
Barley stripe mosaic virus	17 kDa	barley	Gustafson et al., *Nucl. Acids Res.* 14, 3895-3909 (1986)
Potato mop top virus	21 kDa	potato	Scott et al., *J. Gen. Virol.* 75, 3561-3568 (1994)
Peanut clump virus	17 kDa	peanut	Herzog et al., *J. Gen. Virol.* 75, 3147-3155 (1994)
Beet soil-borne virus	22 kDa	Sugar beet	Koenig et al., *Virology* 216, 202-207 (1996)

SEQUENCE LISTING

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<120> METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL SEQUENCE

<130> P.SES.02/WO

<140>
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  1               5                  10                  15

gtt gcc ggt tgt gtt gtt gtc agt atg ttg tac tca ccg ttt ttc agc   96
Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
             20                  25                  30

aac gat gtt aaa gcg tcc agc tat gcg gga gca att ttt aag ggg agc   144
Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
         35                  40                  45

ggc tgt atc atg gac agg aat tcg ttt gct caa ttt ggg agt tgc gat   192
Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
     50                  55                  60

att cca aag cat gta gcc gag tcc atc act aag gtt gcc acc aaa gag   240
Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
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65                  70                  75                  80

cac gat gtt gac ata atg gta aaa agg ggt gaa gtg acc gtt cgt gtt   288
His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
                85                  90                  95

gtg act ctc acc gaa act att ttt ata ata tta tct aga ttg ttt ggt   336
Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
            100                 105                 110

ttg gcg gtg ttt ttg ttc atg ata tgt tta atg tct ata gtt tgg ttt   384
Leu Ala Val Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
        115                 120                 125

tgg tat cat aga taa                                           399
Trp Tyr His Arg
    130
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<223> Description of Artificial Sequence: genetically modified TGB-3 viral sequence

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Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
            20                  25                  30

Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
        35                  40                  45

Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
    50                  55                  60

Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
65                  70                  75                  80

His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
                85                  90                  95

Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
            100                 105                 110
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Trp Tyr His Arg
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1 5 10 15

gtt gcc ggt tgt gtt gtt gtc agt atg ttg tac tca ccg ttt ttc agc 96
Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
20 25 30

aac gat gtt aaa gcg tcc agc tat gcg gga gca att ttt aag ggg agc 144
Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
35 40 45

ggc tgt atc atg gcc gcg aat tcg ttt gct caa ttt ggg agt tgc gat 192
Gly Cys Ile Met Ala Ala Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
50 55 60

att cca aag cat gta gcc gag tcc atc act aag gtt gcc acc aaa gag 240
Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
65 70 75 80

cac gat gtt gac ata atg gta aaa agg ggt gaa gtg acc gtt cgt gtt 288
His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
85 90 95

gtg act ctc acc gaa act att ttt ata ata tta tct aga ttg ttt ggt 336
Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly

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            100                 105                 110

ttg gcg gtg ttt ttg ttc atg ata tgt tta atg tct ata gtt tgg ttt   384
Leu Ala Val Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
        115                 120                 125

tgg tat cat aga taa                                           399
Trp Tyr His Arg
    130
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<210> 4
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<223> Description of Artificial Sequence: genetically modified TGB-3 viral sequence

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Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
             20                  25                  30

Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
         35                  40                  45

Gly Cys Ile Met Ala Ala Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
     50                  55                  60

Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
 65                  70                  75                  80

His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
                 85                  90                  95

Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
            100                 105                 110

Leu Ala Val Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
        115                 120                 125

Trp Tyr His Arg
    130
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Met Val Leu Val Val Lys Val Asp Leu Ser Asn Ile Val Leu Tyr Ile
  1               5                  10                  15

gtt gcc ggt tgt gtt gtt gtc agt atg ttg tac tca ccg ttt ttc agc   96
Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
             20                  25                  30

aac gat gtt aaa gcg tcc agc tat gcg gga gca att ttt aag ggg agc   144
Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
         35                  40                  45

ggc tgt atc atg gac agg aat tcg ttt gct caa ttt ggg agt tgc gat   192
Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
     50                  55                  60

att cca aag cat gta gcc gag tcc atc act aag gtt gcc acc aaa gag   240
Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
 65                  70                  75                  80

cac gat gtt gac ata atg gta aaa agg ggt gaa gtg acc gtt cgt gtt   288
His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
                 85                  90                  95

gtg act ctc acc gaa act att ttt ata ata tta tct aga ttg ttt ggt   336
Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
            100                 105                 110

ttg gat gat ttt ttg ttc atg ata tgt tta atg tct ata gtt tgg ttt   384
Leu Asp Asp Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
        115                 120                 125

tgg tat cat aga taa                                             399
Trp Tyr His Arg
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Met Val Leu Val Val Lys Val Asp Leu Ser Asn Ile Val Leu Tyr Ile
1 5 10 15

Val Ala Gly Cys Val Val Val Ser Met Leu Tyr Ser Pro Phe Phe Ser
20 25 30

Asn Asp Val Lys Ala Ser Ser Tyr Ala Gly Ala Ile Phe Lys Gly Ser
35 40 45

Gly Cys Ile Met Asp Arg Asn Ser Phe Ala Gln Phe Gly Ser Cys Asp
50 55 60

Ile Pro Lys His Val Ala Glu Ser Ile Thr Lys Val Ala Thr Lys Glu
65 70 75 80

His Asp Val Asp Ile Met Val Lys Arg Gly Glu Val Thr Val Arg Val
85 90 95

Val Thr Leu Thr Glu Thr Ile Phe Ile Ile Leu Ser Arg Leu Phe Gly
100 105 110

Leu Asp Asp Phe Leu Phe Met Ile Cys Leu Met Ser Ile Val Trp Phe
115 120 125

Trp Tyr His Arg
130

WHAT IS CLAIMED IS

CLAIMS

1. Method of genetic modification of a TGB-3 wild type viral sequence for reducing or suppressing the possible deleterious effects of the agronomic properties of a transformed plant or plant cell by said TGB-3 viral sequence, comprising the following successive steps :

- submitting said sequence to point mutation(s) which allow the substitution of at least one amino-acid into a different amino-acid,
- selecting genetically modified TGB-3 wild type viral sequences having said point mutation(s) and which are not able to promote cell-to-cell movement of a mutant virus having a dysfunctional TGB-3 wild type viral sequence, when expressed in trans from a replicon,
- further selecting among said genetically modified TGB-3 viral sequences, the specifically genetically modified sequence which inhibits infection with a co-inoculated wild type virus when the mutant form was expressed from a replicon, and
- recovering said specifically genetically modified TGB-3 viral sequence.

2. Method according to claim 1, wherein the TGB-3 wild type viral sequence is the BNYVV P15 sequence.

3. Genetically modified TGB-3 viral sequence obtained by the method according to claim 1 or 2.

4. Genetically modified TGB-3 viral sequence according to claim 3, being selected from the group consisting of the following sequences :

SEQ ID NO 1 :

ATGGTGCTTGTGGTTGCAGTAGCTTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60

M V L V V A V A L S N I V L Y I V A G C

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GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V  V  V  S  M  L  Y  S  P  F  F  S  N  D  V  K  A  S  S  Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
A  G  A  I  F  K  G  S  G  C  I  M  D  R  N  S  F  A  Q  F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G  S  C  D  I  P  K  H  V  A  E  S  I  T  K  V  A  T  K  E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H  D  V  D  I  M  V  K  R  G  E  V  T  V  R  V  V  T  L  T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360
E  T  I  F  I  I  L  S  R  L  F  G  L  A  V  F  L  F  M  I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
C  L  M  S  I  V  W  F  W  Y  H  R  *
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SEQ ID NO 2 :

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ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M  V  L  V  V  K  V  D  L  S  N  I  V  L  Y  I  V  A  G  C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V  V  V  S  M  L  Y  S  P  F  F  S  N  D  V  K  A  S  S  Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGCCGCGAATTCGTTTGCTCAATTT 180
A  G  A  I  F  K  G  S  G  C  I  M  A  A  N  S  F  A  Q  F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G  S  C  D  I  P  K  H  V  A  E  S  I  T  K  V  A  T  K  E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H  D  V  D  I  M  V  K  R  G  E  V  T  V  R  V  V  T  L  T
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GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGCGGTGTTTTTGTTCATGATA 360
E T I F I I L S R L F G L A V F L F M I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
C L M S I V W F W Y H R *

SEQ ID NO 3 :

ATGGTGCTTGTGGTTAAAGTAGATTTATCTAATATTGTATTGTACATAGTTGCCGGTTGT 60
M V L V V K V D L S N I V L Y I V A G C

GTTGTTGTCAGTATGTTGTACTCACCGTTTTTCAGCAACGATGTTAAAGCGTCCAGCTAT 120
V V V S M L Y S P F F S N D V K A S S Y

GCGGGAGCAATTTTTAAGGGGAGCGGCTGTATCATGGACAGGAATTCGTTTGCTCAATTT 180
A G A I F K G S G C I M D R N S F A Q F

GGGAGTTGCGATATTCCAAAGCATGTAGCCGAGTCCATCACTAAGGTTGCCACCAAAGAG 240
G S C D I P K H V A E S I T K V A T K E

CACGATGTTGACATAATGGTAAAAAGGGGTGAAGTGACCGTTCGTGTTGTGACTCTCACC 300
H D V D I M V K R G E V T V R V V T L T

GAAACTATTTTTATAATATTATCTAGATTGTTTGGTTTGGATGATTTTTTGTTCATGATA 360
E T I F I I L S R L F G L D D F L F M I

TGTTTAATGTCTATAGTTTGGTTTTGGTATCATAGATAA 399
C L M S I V W F W Y H R *

5. Vector comprising the genetically modified TGB-3 viral sequence according to the claim 3 or 4, possibly linked to one or more regulatory sequence(s) capable of being active into a plant or a plant cell.

6. Method for inducing resistance into a plant or a plant cell to a virus comprising a TGB-3 sequence, comprising the following steps :

- preparing a nucleic acid construct comprising a genetically modified TGB-3 viral sequence according to claim 4 or 5, being operably linked to one or more regulatory sequence(s) capable of being active into a plant or a plant cell,
- transforming a plant cell with said nucleic acid construct, and possibly
- regenerating a transgenic plant from the transformed plant cell.

7. Method according to claim 6, characterised in that the virus is selected from the group consisting of the apple stem pitting virus, the blueberry scorch virus, the potato virus M, the white clover mosaic virus, the *Cymbidium* mosaic virus, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus, the beet soil-borne virus or the BNYVV virus.

8. Method according to claim 6 or 7, characterised in that the plant cell is a stomatal cell.

9. Method according to any one of the claims 6 to 8, characterised in that the plant is selected from the group consisting of apple, blueberry, potato, clover, orchid, barley, peanut or sugar beet.

10. Method according to any one of the claims 6 to 9, characterised in that the regulatory sequence comprises a promoter sequence or a terminator sequence active in a plant.

11. Method according to claim 10, characterised in that the promoter sequence is a constitutive or a foreigner promoter sequence.

12. Method according to claim 10, characterised in that the promoter sequence is selected from the group consisting of 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin Arabidopsis thaliana promoter.

13. Method according to any one of the claims 10 to 12, characterised in that the promoter sequence is a promoter which is capable of being active mainly into the root tissue of plants such as the par promoter of the haemoglobin gene from Perosponia andersonii.

14. Transgenic plant or transgenic plant cell resistant to a virus and comprising a nucleic acid construct having a genetically modified TGB-3 viral sequence according to claim 4 or 5, being operably linked to one or more regulatory sequence(s) active into a plant or a plant cell.

15. Transgenic plant or transgenic plant cell according to claim 14, characterised in that the virus is selected from the group consisting of the apple stem pitting virus, the blueberry scorch virus, the potato virus M, the white clover mosaic virus, the *Cymbidium* mosaic virus, the potato virus X, the barley stripe mosaic virus, the potato mop top virus, the peanut clump virus, the beet soil-borne virus and the BNYVV virus.

16. Transgenic plant or transgenic plant cell according to claim 14 or 15, being a plant or a plant cell selected from the group consisting of apple, blueberry, potato, clover, orchid, barley, peanut or sugar beet plant or plant cell.

17. Transgenic plant or transgenic plant cell according to any one of the claims 14 to 16, characterised in that the regulatory sequence comprises a promoter

sequence and a terminator sequence capable of being active into a plant.

18. Transgenic plant or transgenic plant cell according to any one of the claims 14 to 17, characterised in that the regulatory sequence(s) comprise a promoter sequence which is a constitutive or a foreigner vegetal promoter sequence.

19. Transgenic plant or transgenic plant cell according to claim 18, characterised in that promoter sequence is selected from the group consisting of 35S Cauliflower Mosaic Virus promoter, and/or the polyubiquitin Arabidopsis thaliana promoter.

20. Transgenic plant or transgenic plant cell according to claim 18 or 19, characterised in that the promoter sequence is a promoter which is mainly active in root tissues such as the par promoter of the haemoglobin gene from Perosponia andersonii.

21. Transgenic plant tissue selected from the group consisting of fruit, stem, root, tuber, seed of a plant according to any one of the claims 14 to 20.

22. Reproducible structure obtained from a transgenic plant according to any one of the claims 14 to 21.

add a3

ABSTRACT

METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL SEQUENCE

The present invention concerns a method of genetic modification of a TGB-3 wild type viral sequence for reducing or suppressing the possible deleterious effects of the agronomic properties of a transformed plant or plant cell by said TGB-3 viral sequence, comprising the following successive steps: submitting said sequence to point mutation(s) which allow the substitution of at least one amino-acid into a different amino-acid; selecting genetically modified TGB-3 wild type viral sequences having said point mutation(s) and which are not able to pormote cell-to-cell movement of a mutant virus having a dysfunctional TGB-3 wild type viral sequence, when expressed in trans from a replicon; further selecting among said genetically modified TGB-3 viral sequences, the specifically genetically modified sequence which inhibits infection with a co-inoculated wild type virus when the mutant form was expressed from a replicon; and recovering said specifically genetically modified TGB-3 viral sequence.

P.SES.02/05

Attorney's Docket No. VANM190.001APC

DECLARATION - USA PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am an original, first and joint inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled METHOD OF GENETIC MODIFICATION OF A WILD TYPE VIRAL SEQUENCE; the specification of which was internationally filed on **July 9, 1999,** as International Application No. **PCT/BE99/00089,** and for which the initial documents for entry into the U.S. National Phase were filed on **January 10, 2001**, and assigned U.S. Serial No. 09/743,905.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56;

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)			Priority Claimed
No.: **98870159.5**	Country: **EUR. PATENT OFFICE**	Date Filed: **July 10, 1998**	**Yes**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

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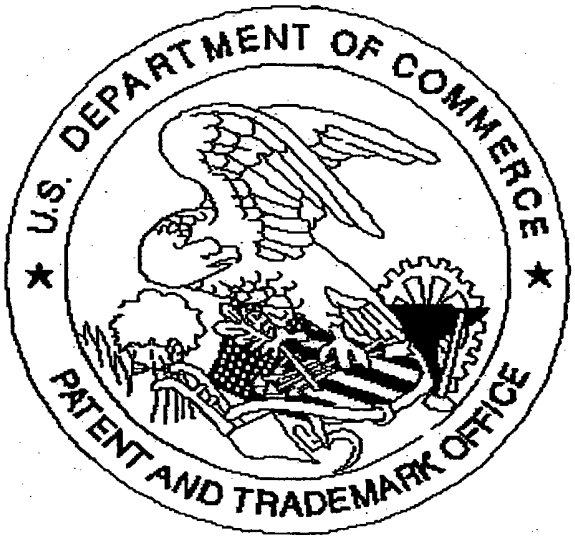

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